インプラント固定に耐え得る新しい生体活性骨セメントの開発

AUTHOR(S):

川那辺, 圭一

インプラント固定に耐え得る新しい生体活性骨セメントの開発

（研究課題番号 15591574）

平成15年度～平成16年度科学研究費補助金

（基盤研究(C)(2)）研究成果報告書

平成17年 5 月

研究代表者 川那辺 圭一

（京都大学医学研究科）

インプラント固定に耐え得る新しい生体活性骨セメントの開発

(研究課題番号　１５５９１５７４)

平成１５年度～平成１６年度科学研究費補助金

(基盤研究(C)(2)) 研究成果報告書

平成１７年５月

研究代表者　川那辺　圭一

(京都大学医学研究科　助教授)

はしがき

研究組織

研究代表者：川那辺　圭一

(京都大学医学研究科　助教授)

交付決定額（配分額）　　(金額単位：千円)

	直接経費	間接経費	合計
平成１５年度	１，７００	０	１，７００
平成１６年度	１，７００	０	１，７００
総計	３，４００	０	３，４００

研究発表

学会発表

後藤公志、田村治郎、新里修一、藤林俊介、川下将一、小久保正、中村孝志
アナタースナノ微粒子含有生体活性骨セメントの開発
日本バイオマテリアル学会
2003.12/16-17

K.Goto, S.Shinzato, S.Fujibayashi, J.Tamura, M.Kawashita, T.Kokubo, T.Nakamura
Osteoconductivity of new bioactive bone cement containing nano-sized TiO2 particles
5th Combined Meeting of the Orthopaedic Research Societies of Canada, U.S.A., Japan and Europe
2004.10/10-13

K.Goto, M.Hashimoto, S.Fujibayashi, K.Kawanabe, T.Kokubo, T.Nakamura
New bioactive bone cement containing nano-sized TiO2 particles
17th International Symposium on Ceramics in Medicine
2004.12/8-12

後藤公志、橋本雅美、藤林俊介、川那辺圭一、小久保正、中村孝志
New bioactive bone cement containing micron sized TiO2 particles
第２４回　整形外科セラミックインプラント研究会
2004. 12/4

出版物

K.Goto, M.Hashimoto, S.Fujibayashi, T.Kokubo, T.Nakamura
New Bioactive Bone Cement Containing Nano-Sized Titania Particles
Key Engineering Materials Vols. 284-286 (2005) p.97-100

はじめに

ポリメチルメタクリレート（PMMA）は、人工関節の固定用や人工骨として整形外科領域の手術において長年にわたって広く用いられている。しかし、PMMA は骨と直接結合することが出来ず、骨の中に埋入した時には骨との間に必ず繊維組織が介在する。人工関節手術において大きな問題であるインプラントの弛みは、PMMA が骨と直接結合できないために生じるという可能性が指摘されている。この問題を解決するために、骨と直接結合できるさまざまな生体活性骨セメントが、近年開発されてきた。われわれの施設でも、ガラスやガラスセラミックスをレジンに分散させたセメントを開発してきたが、ガラスやガラスセラミックスには溶解性の問題があり、人工関節の固定に用いる場合には骨セメントの長期にわたる安定性に懸念があった。

近年優れた生体活性を示すことが判ってきた酸化チタンは、生体親和性に優れ、溶解性が無く、これとポリメチルメタクリレート（ＰMMA）を組み合わせることによって、強度劣化しない長期間安定した生体活性骨セメントを作製することが可能と考えられた。酸化チタンを PMMA に分散させたセメントは、酸化チタンの含有率を高めることによって、その高い X 線不透過性のためにインプラント固定のみならず、椎体などへの骨補填

材料としての応用も期待できる。今回の研究の目的は、酸化チタンを分散させた骨セメントの機械的性質と、生体親和性、骨伝導能等を評価し、臨床応用可能な生体活性骨セメントの開発を進めることにある。

研究成果

酸化チタンの中でも特に生体活性の高いアナタース型の結晶構造を持つφ２００nm の微粒子を PMMA に重量比で５０%加えたセメント（T50c）と、シラン処理した微粒子を５０%及び６０%加えたセメント（ST50c, ST60c）を作成し、その力学的強度および生体内での骨親和性、骨伝導能を調べた。骨親和性、骨伝導能の評価は、ラットの脛骨骨髄内にセメントを埋め込み、周囲の骨との反応を光学顕微鏡、電子顕微鏡等で観察することにより行った。力学的強度に関しては、酸化チタンを含むいずれのセメントもPMMAに対してやや劣っていたが、骨親和性、骨伝導能に関してはセメント埋め込み後６週、１２週の時点において、有意にPMMAより優れており、高い生体活性を持つことが示され、特にST60cは、力学的強度、骨伝導能においてST50cおよびT50cより優れていた。しかし、いずれの酸化チタン含有骨セメントでも、酸化チタンの微粒子が

PMMA 内で凝集体を形成している像が観察され、力学的強度が低い原因となっていると考えられた。そこで、新たに¢2 μm の酸化チタン微粒子をＰＭＭＡに分散させたセメントを開発した。、この微粒子はアナタース型とルチル型の結晶構造をほぼ等量含むもので、高い生体活性を示すものである。この酸化チタン微粒子をシラン処理後、ＰＭＭＡに重量比で 50 % と 56 %加えたセメント（ST2-50c, ST2-56c）を作成し、その力学的強度及び生体内での骨親和性、骨伝道能を調べた。力学的強度に関しては、ST2-50c、ST2-56c とも圧縮強度はＰＭＭＡより優れ、曲げ強度はＰＭＭＡと同等であった。骨伝導能に関しては、セメント埋め込み後６週、１２週、２６週の時点において、ST2-56c が ST2-50c より有意に優れ、¢200 nm の酸化チタン微粒子をＰＭＭＡに分散させたセメント（ST60c）よりも有意に優れていた。

今後の研究

これまでの研究から、¢2 μm の酸化チタン微粒子をＰＭＭＡに分散させたセメントが、力学的強度及び骨伝導能に優れていることが判明したが、ST2-50c、ST2-56c ともに、シラン処理を行っているために、臨床応用す

る際にはシラン処理剤の生体毒性が問題となる可能性がある。最近新たに開発されたϕ 300 nm のルチル型の酸化チタン微粒子は、シラン処理しなくても、PMMA に均一に分散することが判明したため、現在はこの酸化チタン微粒子を PMMA に分散させたセメントを作製して、その力学的強度及び生体内での骨親和性、骨伝道能を調べているところである。さらに、酸化チタン微粒子の含有率を低くして、市販の PMMA 骨セメントと力学的性質が近く、尚且つ生体活性を有する骨セメントの開発も行っていく予定である。

New bioactive bone cement containing nano-sized titania particles

K.Goto,[1,a] M.Hashimoto,[2] S. Fujibayashi,[1] T.Kokubo,[3] T.Nakamura[1]

[1]Department of Orthopaedic Surgery, Faculty of Medicine, Kyoto University, Kawahara-cho 54, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

[2]Japan Fine Ceramics Center, Mutsuno 2-4-1, Atsuta-ku, Nagoya 456-8587, Japan

[3]Research Institute for Science and Technology, Chubu University, 1200 Matsumoto-cho, Kasugai 487-8501, Japan

[a]email: k.g.bau@kuhp.kyoto-u.ac.jp

Keywords: titania; bioactive; osteoconductivity; polymethylmethacrylate

Abstract

Two types of new bioactive polymethylmethacrylate (PMMA)-based bone cements containing nano−sized titania (TiO_2) particles were prepared and evaluated to assess the effect of TiO_2 content on their mechanical properties and osteoconductivity. We prepared two types of bioactive bone cement, ST50c and ST60c, which contained 50 wt% silanized TiO_2 and 60 wt% silanized TiO_2, respectively. Commercially available PMMA cement (PMMAc) was used as a control. The cements were inserted into rat tibiae and solidified in situ. After 6 and 12 weeks, they were taken out for evaluation of osteoconductivity by scanning electron microscopy (SEM), contact microradiography (CMR) and Giemsa surface staining. SEM revealed that ST60c and ST50c apposed to bone directly while PMMAc did not. The affinity index of ST60c was significantly higher than for the other cements at each time interval. The results showed that ST60c was a promising material, but its mechanical strength should be improved before application in prosthesis fixation.

Introduction

PMMA bone cement is generally used for prosthetic fixation clinically. But it cannot bond to bone directly, that causes prosthetic loosening [1]. To overcome the disadvantage we have developed a new type of bioactive bone cement which contains nano-sized titania particles, crystal phase of which is anatase. Recently, anatase has been shown to have excellent apatite forming ability in vitro and osteoconductivity in vivo [2, 3]. Titania is not degraded and is thought to be stable in the body environment. The purpose of this study is to evaluate the osteoconductivity of the titania-containing cements and examining the bone-cement interface histologically.

Materials and Methods

Titanium dioxide powder. Titanium dioxide powder (ISHIHARA SANGYO KAISHA, LTD., Osaka, Japan) with an average particle size of 200 nm was used. Powder X-ray diffraction of the particles showed anatase as its main phase. The powder was mixed into two kinds of TiO_2-dispersed cements designated ST50c and ST60c with 50 and 60 wt%, respectively. For ST50c and ST60c, titanium dioxide particles were treated with γ-methacryloxy propyl trimethoxy silane (Shinetsu Chemical Industry, Tokyo) at 1.0–2.0 wt%, and these silanized particles were subsequently dried and cured at 130 °C for 5 minutes.

Polymethyl methacrylate powder. Spherical PMMA powder, synthesized by suspension polymerization, with an average molecular weight of 270000 and an average particle size of 5 μm (standard deviation: 2 μm) was used [4].

Preparation of the liquid. Liquid methacrylate (MMA) monomer (Wako Pure Chemical Industry, Osaka, Japan) was used.

Cement preparation. Three types of cement, designated ST50c, ST60c and PMMAc, were prepared. PMMAc was a commercialized PMMA-based bone cement (Osteobond, Zimmer, Warsaw, USA) used as a control material. The composition of ST50c and ST60c is shown in Table 1. As an initiator, benzoyl peroxide (Wako Pure Chemical Industry) was added to the powder at 4.0 wt% of the monomer, and as an accelerator, *N,N*-dimethyl-*p*-toluidine (Wako Pure Chemical Industry) was dissolved in the liquid to 2.0 wt% of the monomer. Each cement was prepared by mixing the powder with the liquid for 1 min: polymerization occurred within 7–9 minutes. The mechanical properties of the cured cements are shown in Table 2. These properties were measured using rectangular bars (20 × 4 × 3 mm) of the cement mixtures under dry conditions. After the cement had hardened, the specimens were tested with an Instron-type testing machine (Model AGS-10kNG, Shimazu Co., Kyoto, Japan). The bending strength test was carried out using the 3-point bending method (ASTM F417-78). Eight specimens were used in each mechanical test.

Table 1. Composition of PMMA-based bioactive cements (wt%)

Cement	Powders[a]		Liquid[b]
	TiO_2	PMMA	MMA
ST50c	50	20	30
ST60c	60	16	24

[a]BPO was added in 4 wt% of the MMA

[b]DMPT was added in 2 wt% of the MMA

Table 2. Mechanical properties of ST50c, ST60c and PMMAc (means ± SD, N = 8)

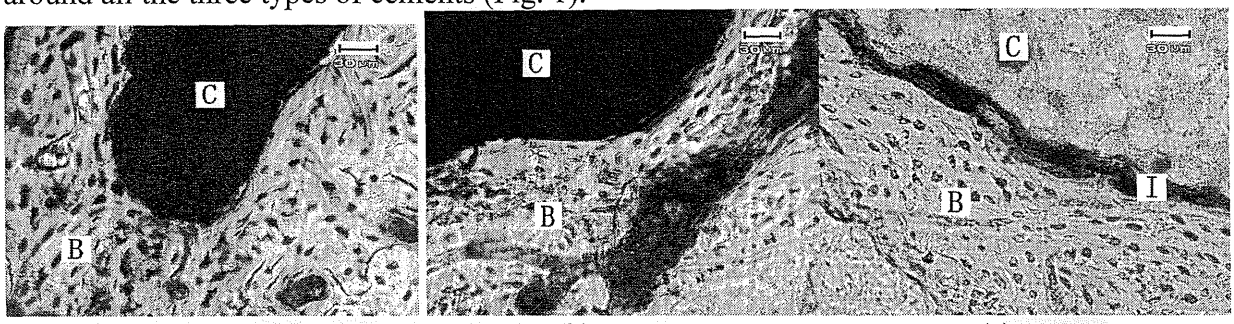
	Bending strength (MPa)	Strain to failure (%)	Bending modulus (GPa)
ST50c	60.5 ± 7.1	1.51 ± 0.23	4.05 ± 0.27
ST60c	52.2 ± 12.5	1.64 ± 0.1	4.23 ± 0.68
PMMAc	106.7 ± 8.0	4.16 ± 0.14	2.56 ± 0.16

Animal experiment. Eight week-old male Wistar rats were used for the implantation study. Cortical bone defects were created at the medial aspect of the proximal metaphysis of both tibiae, and a paste-form cement was inserted into the intramedullary canals of both bone, and each paste was allowed to cure in situ. A total of 18 rats (36 legs) were used, with each of the three types of cements being used in 12 legs. Six legs in each of the three subgroups were sacrificed at 6 and 12 weeks after the operation. The specimens were observed and evaluated using light microscope, contact microradiography (CMR) and scanning electron microscopy (SEM) with an energy-dispersive X-ray micro analyzer (EDX). To evaluate osteoconductivity, affinity indices (%) for each subgroup were calculated by using the SEM photographs.

Statistical analysis. Values were expressed as means and the standard deviations (SD), and values for each cement at each time interval were compared using Fisher's PLSD post hoc statistical test in StatView (version 5.0) for Windows. P values smaller than 0.01 were considered statistically significant.

RESULTS

Evaluation of the bone-cement interface. Giemsa surface staining showed that ST60c was in direct contact with bone without intervening soft tissue in both the 6-week and the 12-week specimens. ST50c also appeared to be in direct contact with bone, but a quite thin layer of soft tissue was suspected to intervene between the cement and bone. There was always a soft tissue layer 10–30 μm wide between PMMAc and bone. Typically, there was no inflammatory reaction around all the three types of cements (Fig. 1).



(a) (b) (c)

Fig. 1. Giemsa surface staining of (a) ST60c, (b) ST50c, (c) PMMAc in rat tibiae 6 weeks after implantation. C, cement; B, bone; I, intervening soft tissue. Bar = 30 μm.

CMR revealed that a wider margin of ST60c and ST50c contacted bone directly than for PMMAc. Remarkably both ST50c and ST60c showed a white line 30–60 μm wide marginally in both the 6- and the 12-week specimens, and that could not be detected in PMMAc (Fig. 2). Low magnification back-scattered SEM revealed that ST60c was in direct contact with bone in large areas within 6 weeks, while ST50c contacted bone directly over small areas. There was always a soft tissue layer between PMMAc and bone. The white line was also seen circumferentially with both ST50c and ST60c. High magnification back-scattered SEM also showed that ST60c bonded to bone directly while PMMAc did not (Fig. 3). SEM-EDX analysis indicated that the white line seen around the titania-containing cements in SEM and CMR photographs mainly represents a Ti rich layer.

Evaluation of osteoconductivity. The affinity indices for all the cements at 6 and 12 weeks, and the statistical comparisons, are shown in Table 3.

Table 3. The affinity indices (%) for ST50c, ST60c and PMMAc in rat tibiae at 6 and 12 weeks after implantation (mean ± S.D., N=12)

	ST50c	ST60c	PMMAc
6w	16.1 ± 10.8	26.8 ± 8.3[a]	8.9 ± 9.4
12w	20.9 ± 7.3	31.3 ± 8.7[a]	14.9 ± 10.6

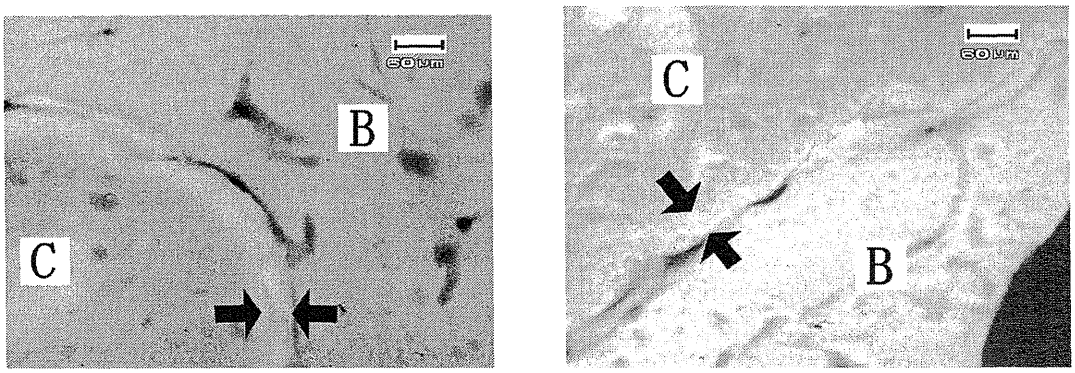
[a]Significant compared with ST50c and PMMAc ($p < 0.01$).

Discussion and Conclusion

According to previous studies, we prepared high molecular weight PMMA for its contribution to the increase of bioactivity of the cement [4]. The TiO_2 contents described above were chosen because cements containing over 60 wt% TiO_2 were difficult to handle and those containing less than 50 wt% TiO_2 had poor in vitro apatite forming ability. Between the titania-containing cements, ST60c showed significantly good osteoconductivity at each time interval compared to ST50c. High percentage of its bioactive titania content is supposed to attribute to superior osteoconductivity of ST60c. CMR and SEM showed that the dispersion of TiO2 in ST50c and ST60c was not adequate and the TiO2 in each cement often aggregated. This aggregation suggests that even after silane-treatment, TiO2 powder tends to aggregate due to its surface interaction. The bending strengths of ST50c and ST60c are inferior to PMMAc, and the strain to

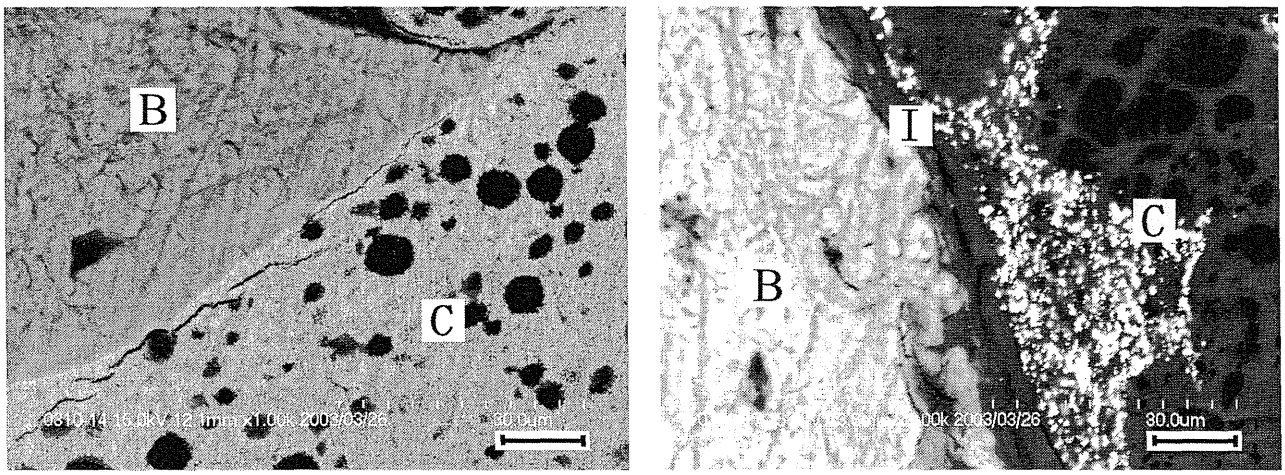
failure and bending modulus of the TiO_2-containing cements indicate that they are more brittle than PMMAc. The mechanical weakness of TiO_2-containing cements is probably due to the inadequate TiO_2 dispersion.

So far titania-containing cements are not promising for prosthetic fixation because of its lower mechanical properties than PMMAc, but they, especially ST60c, are acceptable materials as bone



(a) (b)

Fig. 2. CMR photographs of (a) ST60c and (b) ST50c in rat tibiae 12 weeks after implantation. The white line (between arrows) is usually seen around ST60c and ST50c. C, cement; B, bone. Bar = 60 μm.



(a) (b)

Fig. 3. SEM photographs of ST60c (a), and PMMAc (b) in rat tibiae 6 weeks after implantation. C, cement; B, bone; I, intervening soft tissue. Bar = 10 μm

substitutes since they showed good osteoconductivity and their bending strengths are much stronger than commercially available calcium phosphate cements. Furthermore the handling property of ST60c is much better than the calcium phosphate cements. In conclusion, ST60c is the most acceptable titania-containing cement with good prospects for clinical use due to its good osteoconductivity; however, further research to improve the mechanical strength and to examine the fatigue behavior in vivo should be performed.

[1] Freeman MAR, Bradley GW, Ravell PA: J Bone Joint Surg 1982;64B:489–493.
[2] Uchida M, Kim H-M, Kokubo T, Fujibayashi S, Nakamura T: J. Biomed. Mater. Res 2003;64A:164-170.
[3] Fujibayashi S, Nakamura T, Nishiguchi S, Tamura J, Uchida M, Kim H-M, Kokubo T: J Biomed Mater Res 2001; 56: 562–570.

[4] Nakamura T, Kato H Okada Y, Shinzato S, Kawanabe K, Tamura J, Kokubo T: In: Giannini S, Moroni A, editors. Bioceramics, Vol. 13. Bologna, Italy: Trans Tech;2000. p 661–664.

チタニア微粒子含有生体活性骨セメントの開発

Development of PMMA-based bioactive bone cements containing titania particles

京都大学医学部

○ 後藤公志、藤林俊介、川那辺圭一、中村孝志

(財)ファインセラミックスセンター

橋本雅美

中部大学総合工学研究所

小久保正

Graduate School of Medicine, Kyoto University

Koji Goto, Shunsuke Fujibayashi, Keiichi Kawanabe, Takashi Nakamura

Japan Fine Ceramics Center

Masami Hashimoto

Research Institute for Science and Technology, Chubu University

Tadashi Kokubo

abstract

To realize an ideal bone cement for percutaneous vertebroplasty (PVP), we have developed PMMA-based bioactive bone cements which contained titania particles, and evaluated on their mechanical properties, setting properties, biocompatibility and osteoconductivity. The results showed that they were promising materials as bone substitutes for PVP.

はじめに

経皮的椎体形成術（PVP）に用いられる骨セメントは以下のような特性を持つことが望ましい。すなわち、高いX線不透過性、ある程度の機械的強度を術中もしくは術直後から獲得できる硬化特性、生体親和性、優れた骨伝導能などである。そのような骨セメントを実現するため、我々はチタニア粒子をポリメチルメタクリレート（PMMA）に分散させた生体活性骨セメントを開発してきた。チタニアはその結晶構造によっては、高い生体活性を有することが近年報告されている[1,2]。この研究の目的は、チタニア粒子を含有する生体活性骨セメントの機械的強度、硬化特性、生体親和性および骨伝導能を評価することにある。

材料および実験方法

セメントの作製

チタニアは、平均粒子径が 0.2 μm と 2 μm の２種類のパウダー状の粒子を準備した。それぞれの粒子を粉末 X 線解析にて調べたところ、¢0.2 μm のものはアナタースで、¢2 μm のものは、アナタースとルチルをほぼ等量含んでいた。

PMMA は分子量 270000、平均粒子径が 5 μm のものを使用した[3]。¢2 μm のチタニアを重量率で 50 %、55.6 %含むセメントを作製し、それぞれ ST2-50c、ST2-56c と名付けた。同様に、¢0.2 μm のチタニアを 50 %、60 %含むセメントを作製し、それぞれ ST0.2-50c、ST0.2-60c と名付けた。それぞれのセメントの組成を表１に示す。それぞれの骨セメントはパウダーとリキッドを約１分間混ぜて準備した。

機械的強度の測定

それぞれの骨セメントの圧縮強度、曲げ強度及び曲げ弾性率を、ISO 5833 の基準に基づいて測定した。圧縮強度の測定は直径 6 mm、長さ 12 mm の円柱を用いて行い、曲げ強度の測定では、20 × 70 × 5 mm の直方体を用いて４点曲げ試験を行った。

硬化時間および温度

内径 6mm、高さ 6mm のテフロンモールド内にセメントを流し込み、気温 23℃、湿度 54~65%の下で測定を行った。セメント硬化時の温度を 10 秒毎に赤外放射温度計にて測定し、時間経過と温度の関係から、硬化時間を ISO 5833 の基準に沿って算出した。

動物実験

８週齢の Wistar rat を用い骨セメントの埋入試験を行った[4-6]。脛骨近位部内側の皮質骨に、幅 2 mm、長さ 7 mm の骨孔を作成し、そこからペースト状のセメントを用手的に埋入して硬化させた。総計２４匹（４８肢）のラットを用い、４種類の骨セメントをそれぞれ１２肢に埋入した。各セメントを埋入された１２匹（２４肢）を、手術後６週、１２週でそれぞれ屠殺した。屠殺後の試料はエタノールにて段階的に脱水してエポキシ樹脂に包埋し、脛骨の骨軸に垂直に薄切して組織学的観察のための標本作成を行った。それぞれの標本の電子顕微鏡写真から affinity index[7] を算出し、骨伝導能の評価を行った。

統計解析

機械的強度、affinity index の結果に関して分散解析を行い、有意水準を $p < 0.01$ とした。

結果

機械的強度

結果を図１に示す。ST2-50c, ST2-56c が ST0.2-50c, ST0.2-60c に比べ、圧縮強度、曲げ強度、曲げ弾性係数とも有意に高かった。また、圧縮強度に関しては ST2-56c が ST2-50c に比べて有意に高かった。全体に ST2-50c, ST2-56c の方が、ST0.2-50c, ST0.2-60c に比べデータのばらつきが少ない傾向が見られた。

硬化時間及び温度 (peak temperature)

硬化時間については、ST2-50c が９分、ST2-56c が８分５０秒、ST0.2-50c が１０分１０秒、ST0.2-60c が９分１０秒であった。Peak temperature については ST2-50c が 93℃、ST2-56c が 81℃、ST0.2-50c が 92℃、ST0.2-60c が 79℃であった。

骨親和性、及び骨伝導能

いずれのセメントについても、ギムザ染色を行った光学顕微鏡による観察において、セメント周囲での有意な炎症所見は認めず、骨との接触（bone apposition）が部分的に確認できた。電子顕微鏡による観察において、ST2-56c は他のセメントに比べ、有意に骨と直接結合している領域が大きく、ST2-50c と ST0.2-50c に関しては、骨との界面に数ミクロンの繊維組織の介在を認めることが多かった。また、ST0.2-50c, 60c に関しては、セメント内部に数十から数百ミクロン単位のチタニアの凝集体を認めることが多かったが、ST2-50c, 56c では稀であった。反射電子像では、いずれのセメントにおいても、その辺縁部に、骨との接触に関係なく 30 ~ 60 μm の'white line'を認めた。Affinity index による骨伝導能の評価を図２に、その評価に用いた代表的な低倍率の電子顕微鏡写真を、それぞれのセメントについて図 3 に示す。Affinity index は、６週、１２週とも ST2-56c が他のセメントと比較して有意に高かった。

考察

今回の実験で、機械的強度に関しては、ST2-50c, 56c が ST0.2-50c, 60c に比べて有意に高く、特に曲げ強度はそれらの 3 倍に達していた。動物実験でも明らかになったように、¢0.2 μm のチタニアは 2 μm のチタニアよりセメント内部で凝集する傾向があり、曲げ試験では、チタニアの凝集体の有無の影響が大きかったものと推察される。セメントの硬化時間に関しては、ST0.2-50c, 60c の方が長い傾向があり、¢0.2 μm のチタニアの方が小さい為に PMMA と MMA および MMA 間に介在して、重合反応を遅らせた可能性があると考えられた。peak temperature に関しては ST0.2-60c, ST2-56c で低い傾向を認め、重合熱を発生させるモノマーの含有量が少ない為に、peak temperature が低くなったものと考えられた。埋入試験では、ST2-56c が最も骨伝導能に優れていることが示された。チタニアがより均一に PMMA 内で分散された結果、セメント辺縁部の安定性が向上したことと、ST2-56c では 2 μm のチタニアがセメント表面により多く表出した為に、骨伝導能を向上させたものと考えられる。White line に関しては、元素分析の結果からチタニアの辺縁部への集積が原因となっていると考えられた。これは PMMA と比較して親水性の高い生体活性チタニアが、セメントが硬化時に血液と混ざることによって辺縁部に移行した可能性があると考えられた。現在、経皮的椎体形成術で実際に用いられている骨セメントは、主に市販の PMMA セメントにＸ線不透過の物質を加えて少し改良したものが一般的であるが、高い重合熱やモノマーの毒性、加えて骨伝導能の欠如が合併症を引き起こす可能性が指摘されている[8-10]。しかし、PMMA 骨セメントを用いた場合のように、術直後から高い強度が獲得できることが望ましい。というのも圧迫骨折した椎体は不安定であり、骨セメントが硬化していない状態では、術後の動作次第で骨折部の変形が進行する恐れが高い為である。高齢者においては、長期臥床の合併症がしばしば問題となることから、術後早期から歩行練習を行うことが重要であり、それには骨折椎体を支えるだけの強度が必要となる。我々が開発したチタニア微粒子含有生体活性骨セメントは、PMMA の欠点を抑え、優れた骨伝導能と高い機械的強度を早期に獲得できることから、PVP 用の骨セメントとして有望であると考えられる。

Captions

表１　Composition

図１　Mechanical properties

図２　Affinity index

図３　SEM photographs of a) ST0.2-50c, b) ST0.2-60c, c) ST2-50c, and d) ST2-56c in rat tibiae 6 weeks after implantation.

1. Uchida M, Kim HM, Kokubo T, Fujibayashi S, Nakamura T. Structural dependence of apatite formation on titania gels in a simulated body fluid. J Biomed Mater Res 2003;64A(1):164-70.
2. Fujibayashi S, Nakamura T, Nishiguchi S, Tamura J, Uchida M, Kim HM, Kokubo T. Bioactive titanium: effect of sodium removal on the bone-bonding ability of bioactive titanium prepared by alkali and heat treatment. J Biomed Mater Res 2001;56(4):562-70.
3. Nakamura T, Kato H, Okada Y, Shinzato S, Kawanabe K, Tamura J, Kokubo T. Bone cement made of high molecular weght PMMA resin with bioactive filler showed higher bone-bonding strength than that of Bis-GMA resin and bioactive ceramic fillers. In: Giannini S, Moroni A, editors. Bioceramics. Bologna, Italy: Trans Tech; 2000. p 661-664.
4. Tamura J, Kawanabe K, Yamamuro T, Nakamura T, Kokubo T, Yoshihara S, Shibuya T. Bioactive bone cement: the effect of amounts of glass powder and histologic changes with time. J Biomed Mater Res 1995;29(5):551-9.
5. Kobayashi M, Shinzato S, Kawanabe K, Neo M, Matsushita M, Kokubo T, Kikutani T, Nakamura T. Alumina powder/Bis-GMA composite: effect of filler content on mechanical properties and osteoconductivity. J Biomed Mater Res 2000;49(3):319-27.
6. Shinzato S, Kobayashi M, Mousa WF, Kamimura M, Neo M, Kitamura Y, Kokubo T, Nakamura T. Bioactive polymethyl methacrylate-based bone cement: comparison of glass beads, apatite- and wollastonite-containing glass-ceramic, and hydroxyapatite fillers on mechanical and biological properties. J Biomed Mater Res 2000;51(2):258-72.
7. Hayashi K, Uenoyama K, Matsuguchi N, Nakagawa S, Sugioka Y. The affinity of bone to hydroxyapatite and alumina in experimentally induced osteoporosis. J Arthroplasty 1989;4(3):257-62.
8. Dahl OE, Garvik LJ, Lyberg T. Toxic effects of methylmethacrylate monomer on leukocytes and endothelial cells in vitro. Acta Orthop Scand 1994;65(2):147-53.
9. Deramond H, Wright NT, Belkoff SM. Temperature elevation caused by bone cement polymerization during vertebroplasty. Bone 1999;25(2 Suppl):17S-21S.
10. Leeson MC, Lippitt SB. Thermal aspects of the use of polymethylmethacrylate in large metaphyseal defects in bone. A clinical review and laboratory study. Clin Orthop

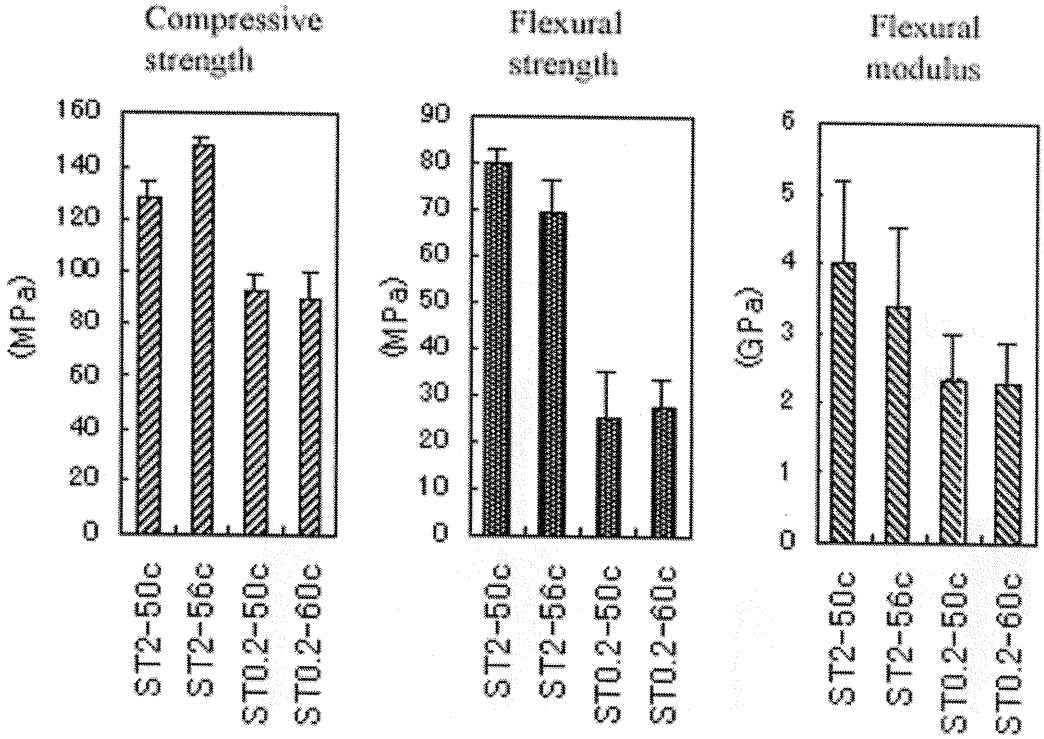
1993(295):239-45.

表1

Cement	Powders (a)		Liquid (b)
	Titania	PMMA	MMA
ST2-50c	50	16.7	33.3
ST2-56c	55.6	14.8	29.6
ST0.2-50c	50	20	30
ST0.2-60c	60	16	24

(a) BPO was added in 4 wt% of the methyl methacrylate (MMA).
(b) DMPT was added in 2 wt% of the MMA.

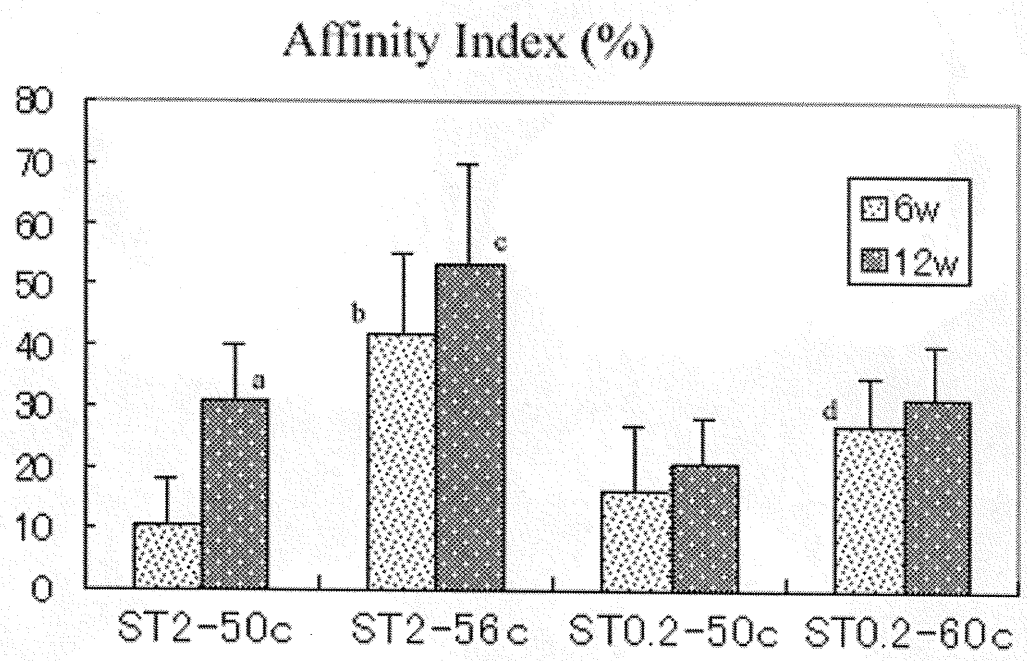
図1



	Compressive strength (MPa)	Flexural strength (MPa)	Flexural modulus (GPa)
ST2-50c	127.9 ± 6.4	80.3 ± 3.0	3.88 ± 0.46
ST2-56c	147.7 ± 3.2	69.3 ± 7.4	4.07 ± 0.84
ST0.2-50c	91.8 ± 7.7	25.5 ± 9.5	2.34 ± 0.63
ST0.2- 60c	89.2 ± 10.6	27.5 ± 5.7	2.24 ± 0.62

Mean ± S.D. N = 5

図2



6w	10.6±7.8	42.1±12.9	16.1±10.8	26.8±8.3
12w	30.8±9.0	53.4±16.6	20.9±7.3	31.3±8.7

Values are expressed as mean±S.D.. N=12.

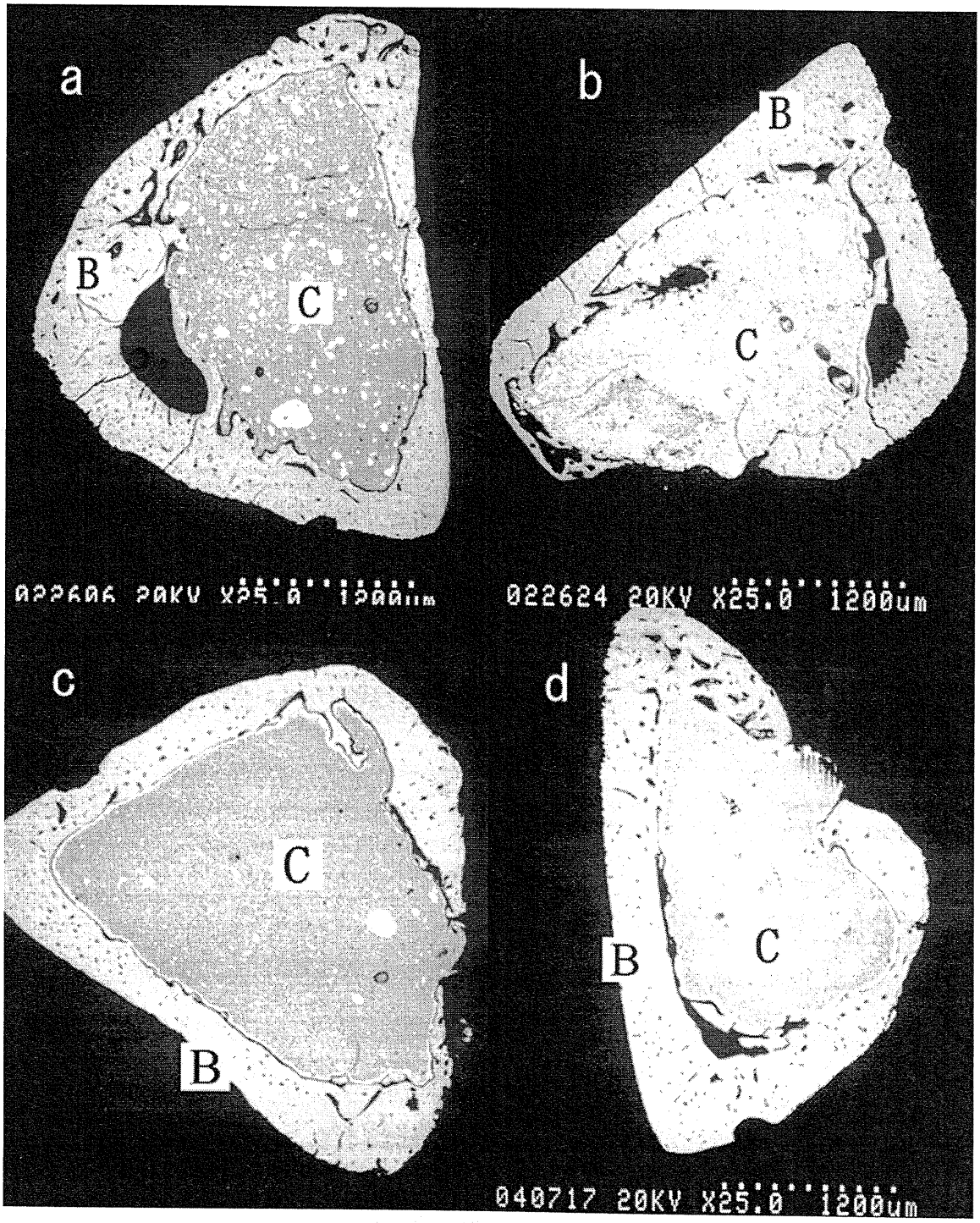
a) significantly greater than at 6 weeks.

b) significantly greatrer than ST2-50c, ST0.2-50c at 6 weeks.

c) significantly greater than ST2-50c, ST0.2-50c, and 60c at 12 weeks.

d) significantly greater than ST2-50c at 6 weeks.

図3


a
B
C
b
B
C
022624 20KV X25.0 1200um
c
C
B
d
B
C
040717 20KV X25.0 1200um

Bioactive bone cements containing nano-sized titania particles for use as bone substitutes

K.Goto,[1,*] J.Tamura,[1] S.Shinzato,[1] S.Fujibayashi,[1] M.Hashimoto,[2]

M. Kawashita, [3] T.Kokubo,[4] T.Nakamura[1]

[1]Department of Orthopaedic Surgery, Faculty of Medicine, Kyoto University, Kawahara-cho 54, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

[2]Japan Fine Ceramics Center, Mutsuno 2-4-1, Atsuta-ku, Nagoya 456-8587, Japan

[3]Faculty of Engineering, Kyoto University, KyotoDaigaku-Katsura, Nishigyo-ku, Kyoto 615-8510, Japan

[4]Research Institute for Science and Technology, Chubu University, 1200 Matsumoto-cho, Kasugai sub487-8501, Japan

Abbreviated title: Titania-particle cement as bone substitute

* Corresponding Author:

Koji Goto, M.D.

Department of Orthopaedic Surgery, Faculty of Medicine, Kyoto University, Kawahara-cho 54, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

TEL +81-75-751-3365 FAX +81-75-751-8409

e-mail: k.g.bau@kuhp.kyoto-u.ac.jp.

Abstract

Three types of bioactive polymethylmethacrylate (PMMA)-based bone cement containing nano-sized titania (TiO_2) particles were prepared, and their mechanical properties and osteoconductivity evaluated. The three types of bioactive bone cement were T50c, ST50c, and ST60c, which contained 50 wt% TiO_2, and 50 and 60 wt% silanized TiO_2, respectively. Commercially available PMMA cement (PMMAc) was used as a control. The cements were inserted into rat tibiae and allowed to solidify in situ. After 6 and 12 weeks, tibiae were removed for evaluation of osteoconductivity using scanning electron microscopy (SEM), contact microradiography (CMR), and Giemsa surface staining. SEM revealed that ST60c and ST50c were directly apposed to bone while T50c and PMMAc were not. The osteoconduction of ST60c was significantly better than that of the other cements at each time interval, and the osteoconduction of T50c was no better than that of PMMAc. The compressive strength of ST60c was equivalent to that of PMMAc. These results show that ST60c is a promising material for use as a bone substitute.

Keywords: Titanium oxide; Bioactivity; Osteoconduction; Polymethylmethacrylate

1. Introduction

It is more than 40 years since Charnley first used polymethylmethacrylate (PMMA) for fixation of prostheses [1], and PMMA is now widely used in orthopedics for prosthesis fixation. However, it has been shown that PMMA cannot bond to bone directly: an intervening soft tissue layer usually exists between the bone and the cement, which occasionally leads to aseptic loosening [2,3]. On the other hand, PMMA has been demonstrated to be biocompatible and easy to shape in vivo, allowing its use as a bone substitute in reconstructive surgery of the knee [4] and in vertebroplasty [5–8]. However, the strongly exothermic setting reaction, the toxic effects of the monomer and, again, the inability to bond directly to bone, pose several potential risks [9–11]. Indeed, clinical complications have been reported in vertebroplasty [12,13], and these complications could have been avoided if the cements used had the ability to be well integrated and bind to bone, or if the monomer content of PMMA bone cement had been so reduced that the thermal and toxic effects were decreased.

To overcome these problems, many types of bioactive bone cements have been developed [14]. Among these cements, we have focused on composites of polymers and bioactive materials and, as a result, we have developed several bioactive bone cements [15–19]. In this process, we revealed that the use of bioactive fillers of small particle size and high molecular weight PMMA powder favorably affected the bioactivity of the PMMA-based cements [19,20].

Recently, nano-sized powders of anatase, a crystal phase of titania, have been successfully manufactured. Anatase has been shown to have excellent in vitro apatite-forming ability [21] and in vivo osteoconductivity [22]. Therefore, PMMA-based bone cement containing anatase titania powder may have superior bioactivity and may

be suitable for application as a bone substitute. The purpose of this study was to evaluate the mechanical properties and osteoconductivity of titania-containing cements by performing mechanical tests, examining affinity indices in rat tibiae, and histologically examining the bone–cement interface.

2. Materials and Methods

2.1. Preparation of cement precursors

Titania powder (Ishihara Sangyo Kaisha Ltd, Osaka, Japan) with an average particle size of 200 nm and a specific surface area of 9.5 m^2/g was used. Powder X-ray diffraction of the particles showed anatase as the main phase (Fig. 1).

The powder was mixed with PMMA powder to give three kinds of TiO_2-dispersed cements, designated T50c, ST50c, and ST60c with 50, 50, and 60 wt% TiO_2, respectively. For ST50c and ST60c, the titania particles were treated with γ-methacryloxypropyltrimethoxysilane (Shinetsu Chemical Industry, Tokyo, Japan) at 1.0–2.0 wt%; the silanized particles were subsequently dried and cured at 55 °C for 5 h.

Spherical PMMA powder, synthesized by suspension polymerization [23] with an average molecular weight of 270,000 daltons and an average particle size of 5 μm (standard deviation: 2 μm) [24] was used.

Liquid methylmethacrylate (MMA) monomer (Wako Pure Chemical Industries, Osaka, Japan) was used.

2.2. Cement preparation

Four types of cement, designated T50c, ST50c, ST60c, and PMMAc, were prepared. PMMAc was a commercially available PMMA-based bone cement

(Osteobond; Zimmer, Warsaw, USA) and was used as a control material. The compositions of each cement containing TiO_2 are shown in Table 1. As an initiator, benzoyl peroxide (Wako Pure Chemical Industries) was added to the powder at 4.0 wt% of the monomer and, as an accelerator, *N,N*-dimethyl-*p*-toluidine (Wako Pure Chemical Industries) was dissolved in the liquid to 2.0 wt% of the monomer. Each cement was prepared by mixing the powder with the liquid for 1 min. The setting times which were measured using a Vicat needle in the central part of a Teflon mold (inner diameter 6 mm, height 6 mm) filled with cement, were 8 min 20 s for T50c, 8 min 10 s for ST50c, and 7 min 20 s for ST60c.

2.3. Mechanical testing

The compressive strength, bending strength, and bending modulus of each cement were measured using five specimens in each mechanical test. For bending mechanical analysis, four-point bend testing was performed. The specimens were cut to the desired shape and then polished, using 400 grit silicon carbide paper, to a size of 70 mm × 20 mm × 5 mm. A testing machine, Model 5582 (Instron, Nagoya, Japan), was used to apply a load. The span between outer loading points was 60 mm, and 20 mm between the outer and inner loading points. Measurements were performed with a cross-head speed of 5.0 mm/min at room temperature in air, according to ISO 5833. Bending modulus and bending strength were calculated as:

$$\text{Bending strength} \quad \sigma = (3p_fL/bd^2) \tag{1}$$

$$\text{Bending modulus } E = \delta L \times (3l^2 - 4L^2/4fbd^3), \tag{2}$$

where p_f was the load at fracture (N), L was the distance between the inner and outer loading points (millimeters), b was the sample width (millimeters), d was the sample height (millimeters), l was the distance between outer loading points (millimeters), f

was the difference between the deflections under the loads of 15 and 50 N (millimeters), and δ was the load range. For compressive mechanical analysis, specimens had dimensions of 12 mm length and 6 mm diameter. They were polished, using 400 grit silicon carbide paper, to remove defects from their surfaces. The strength measurement was carried out at a cross-head speed of 20 mm/min according to ISO 5833. The tests were carried out at room temperature in air. The compressive strength was calculated from the following equation:

$$\text{Compressive strength } \sigma_f = F/A, \qquad (3)$$

where F was fracture load (N) and A was the initial cross-sectional area (mm^2).

2.4. Animal experiments

Eight-week-old male Wistar rats weighing 180–230 g were used for the implantation study, following guidelines for use of experimental animals set by Kyoto University, Japan. The animals were husbanded and experiments were performed at the Institute of Laboratory Animals, Faculty of Medicine, Kyoto University. The rats were operated on under general anesthesia induced by intraperitoneal injection of sodium 5-ethyl-5-(1-methylbutyl) barbiturate (sodium pentobarbitone (Nembutal); Dainippon Pharmaceutical Co., Osaka, Japan) at 40 mg/kg of body weight. For evaluation of osteoconductivity, cortical bone defects measuring 2 mm × 7 mm were created in the medial aspect of the proximal metaphyses of both tibiae, and bone marrow was curetted. The intramedullary canals of the bone defects were irrigated with physiological saline, paste-form cement was inserted at random, and each paste was allowed to cure in situ [15–19]. Titania-containing cement powders were milled manually before the animal experiments. Twenty-four rats (48 legs) were used, with

each type of cement being used in 12 legs. Three mice (six legs) from each of the four subgroups were killed 6 and 12 weeks after the operation.

2.5. Microscopic examination

Specimens were dehydrated through a graded ethanol series (70, 80, 90, 99, and 100 vol%) and embedded in epoxy resin (Epofix; Struers Co., Copenhagen, Denmark). Using a band saw (BS-3000; EXAKT, Norderstedt, Germany), thin sections (100 or 500 μm in thickness) were cut perpendicular to the axis of a tibia containing the cement. We could typically make five sections from each leg. The third section (100 μm in thickness) from the most distal portion of each leg was ground to a thickness of 60–80 μm using a grinding–sliding machine (Microgrinding MG-4000; EXAKT) for Giemsa surface staining. The second and fifth sections (100 μm in thickness) from each leg were prepared for contact microradiography (CMR). The first and fourth sections (500 μm in thickness) from each leg were polished with diamond paper and coated with a thin layer of carbon for observation using a scanning electron microscope (SEM: S-4700; Hitachi Ltd, Tokyo, Japan); some of these SEM specimens were analyzed using an energy-dispersive X-ray micro analyzer (EMAX-7000; Horiba Ltd, Kyoto, Japan) attached to the SEM (S-4700).

To evaluate osteoconductivity, affinity indices, as percentages, were calculated for each subgroup from the 12 SEM photographs of the tibial sections from the six tibiae in the group (i.e., two sections from each tibia in each subgroup). To calculate the affinity index [25] from an SEM photograph, we divided the length of bone in direct contact with the cement surface without any intervening soft tissue by the total length of the cement surface, and this value was multiplied by 100 [15–19,23]. The length was measured using an integrated image analyzer (Tectron, Kyoto, Japan) [15–19,23].

To examine only the reaction of the cement within the bone, the area of the cortical bone defect was excluded when the total length of the cement surface was measured.

2.6. Statistical analysis

Values were expressed as means and standard deviations (SD), and values for each cement at each time interval were compared using one-way analysis of variance. Subsequently, possible differences were investigated using Fisher's PLSD post hoc statistical test using StatView (version 5.0) for Windows. A p value less than 0.01 was considered statistically significant.

3. Results

3.1. Mechanical properties

The results of mechanical property determinations and typical stress-strain curves of compressive and bending strength tests are shown in Fig. 2. The ultimate compressive strengths of T50c, ST50c, ST60c, and PMMAc were 70.3 ± 9.7, 91.8 ± 7.7, 89.2 ± 10.6, and 87.9 ± 2.7 MPa, respectively. The ultimate bending strengths of T50c, ST50c, ST60c, and PMMAc were 34.4 ± 4.5, 25.5 ± 9.5, 27.5 ± 5.7, and 59.4 ± 7.8 MPa, respectively. The bending moduli of T50c, ST50c, ST60c, and PMMAc were 2.80 ± 0.70, 2.37 ± 0.63, 2.24 ± 0.62, and 1.56 ± 0.28 GPa, respectively.

3.2. Evaluation of the bone–cement interface

Giemsa surface staining showed that there was almost no inflammatory reaction around any of the cement types in both the 6-week and the 12-week specimens (Fig. 3). Between the 6-week and the 12-week specimens no significant change in appearance could be seen with Giemsa surface staining. While ST60c was in direct

contact with bone with no intervening soft tissue in large areas (Fig. 3c,g), ST50c and T50c also appeared to contact bone directly, but a thin layer of soft tissue was suspected to be present between the cement and bone (Fig. 3a,b,e,f). Furthermore, T50c often had clefts in it (Fig. 3a,e). There was always a soft tissue layer 10–30 μm wide between PMMAc and bone (Fig. 3d,h).

CMR revealed that a wider margin of ST60c and ST50c contacted bone directly than for T50c and PMMAc (Fig. 4a–d). Remarkably, all three types of cement containing titania showed a marginal white line 30–60 μm wide in both the 6- and the 12-week specimens, which was not detected around PMMAc. The presence of this white line was independent of bone apposition, and it always existed in large areas whether the cement contacted bone or fibrous tissue (Fig. 4a–c). The white line in ST60c-containing samples was more clearly observed than those in T50c- and ST50c-containing samples. The contact areas shown by CMR were typically wider than seen by SEM. CMR also revealed some micron-sized aggregates in T50c, ST50c, and ST60c, but there was no significant difference among those cements in the number and size of the aggregates.

Back-scattered SEM revealed that ST60c was in direct contact with bone in relatively large areas within 6 weeks (Fig. 5c), while ST50c contacted bone directly over small areas; frequently there was a soft tissue layer less than 10 μm in thickness between ST50c and the bone (Fig. 5b). There was always a soft tissue layer between T50c and PMMAc and the bone (Fig. 5a,d). The intervening fibrous tissue layer between ST50c or ST60c and bone in the areas where the cement did not contact bone, was always thinner than that between PMMAc and bone.

Back-scattered SEM and SEM-EDX demonstrated that ST60c bonded to bone directly (Fig. 6a,b). The white line was as clear in the SEM photographs as in the

CMR photographs; however, EDX analysis showed a Ti rich layer at the rim of ST60c (Fig. 7).

3.3. Evaluation of osteoconductivity

The affinity indices for all the cements at 6 and 12 weeks, and the statistical comparisons, are shown in Fig. 8. The values for T50c, ST50c, ST60c, and PMMAc were 10.7 ± 7.3, 16.1 ± 10.8, 26.8 ± 8.3, and 8.9 ± 4.4, respectively, at 6 weeks, and 11.0 ± 5.5, 20.9 ± 7.3, 31.3 ± 8.7, and 14.9 ± 10.6, respectively, at 12 weeks.

4. Discussion

Titania is spontaneously formed on titanium in air and electrolytes; it is stable in the body and does not degrade. Recently Uchida et al. [21] reported that titania with specific crystal structures such as anatase are effective in apatite formation in vitro, which is believed to be a prerequisite for bioactivity. Cements containing titania have already been applied in endodontics as a root canal sealer, where the aim of including titania is to control setting time and handling of the cement [26]. We are the first to use titania to add bioactivity to bone cement. Our preliminary studies revealed that PMMA-based composite cements containing over 60 wt% titania or 70 wt% silanized titania powder were difficult to make, because of limitations in filler loading, especially with cement containing nonsilanized titania powder: silane treatment of filler particles reduces the amount of monomer required to achieve sufficient wetting of the filler particles [27]. It was also revealed that the more titania powder the cements contained, the better the apatite-forming ability of the cement in simulated body fluid [28], although there was no significant difference on the apatite-forming ability between cements containing silanized and nonsilanized titania powders.

Shinzato also reported that there was a trend for the osteoconductivity of PMMA-based bioactive bone cements to increase as bioactive filler content increased up to 70 wt% [23]. Consequently, we chose T50c, ST50c, and ST60c as promising materials for this in vivo study. In accordance with our previous study [19], we used high molecular weight PMMA powder because it showed low solubility in the MMA monomer in the polymerizing reaction. As a result, titania could be exposed at the cement surface without being covered by a layer of polymerized MMA, which presumably would contribute to the osteoconductivity.

In the present study, ST60c showed significantly increased osteoconductivity at each time interval when compared with PMMAc. Among the titania-containing cements, ST60c showed significantly increased osteoconductivity at each time point compared with T50c and ST50c, while ST50c did so only at 12 weeks compared with T50c. The high percentage of bioactive titania content is thought to contribute to the superior osteoconductivity of ST60c. T50c showed less osteoconductivity than ST50c, possibly because nonsilanized titania particles in T50c could not disperse well and polymerization of MMA was incomplete, especially near the cement surface; consequently the T50c collapsed gradually and often formed clefts, disturbing bone apposition, as revealed by Giemsa surface staining. Moreover, leakage of the nonpolymerized MMA monomer from the cement surface of T50c may have contributed to the decrease in its osteoconductivity.

As revealed by histological examination, all the cements containing titania particles had good biocompatibility and there was no evidence of the detachment of titania particles from the cement surface. There is always a concern about particle detachment, which could elicit an inflammatory response [29], and further

experiments on long-term local and systemic responses to the cement implantation should be performed.

The white line seen around the titania-containing cements in CMR photographs mainly represents a Ti rich layer, as indicated by SEM-EDX analysis. The formation of a Ti rich layer at the rim of T50c, ST50c, and ST60c is thought to be because anatase-titania, which has abundant Ti–OH groups on its surface [21,30], is relatively hydrophilic in comparison with methylmethacrylate and it takes several minutes for the cement inserted into rat tibiae to solidify completely. No compression was applied while the cement was setting, so some blood mixed with the cement at the rim and, as a result, titania particles possibly gathered at the rim to form a Ti-rich layer. However, the possibility that the release of nonreacted monomer might cause the dense titania-containing layer and thus form the white line could not be ruled out. In the present study, histologically, the intensity of the white line appeared not to decrease and bone apposition along the line was not disturbed. It is not known whether the titania lines positively affect the bone-bonding strength of cement. This should be confirmed using a bonding strength test. However, the histological findings indicated the titania lines at the interface did not have a negative effect on the biocompatibility of cement, and that they promoted contact between the bone and the cement.

The compressive strengths of ST50c and ST60c were equivalent to PMMAc, while that of T50c was significantly lower than PMMAc. As Beatty et al. [31] noted, when fillers are added to an unfilled matrix without coupling, the stress-bearing cross-sectional area of the matrix is reduced. Our data supports this hypothesis. In contrast, the bending strengths of ST50c and ST60c were significantly lower than that of PMMAc. Bending tests generate tensile stress in the cement sample, and the stress concentrates in cracks to break down the sample rapidly, while compressive stress

does not. There is no binding energy between aggregated titania powder particles, whether silane treated or not. In this experiment, tensile stress in the cement was presumably concentrated in the cracks in the aggregates and, as a result, the bending strength of cements containing titania was decreased. Silanized particles tend to aggregate more than nonsilanized particles, as was reported by Wang et al. [32], which might contribute to the lower bending strengths of ST50c and ST60c compared with that of T50c seen in this study. The powder preparation for mechanical testing included no milling process, so the nano-sized titania powder might aggregate more readily than that used in the animal experiments, in which milled powders were used.

Similar types of PMMA-based composite cement were developed by Shinzato et al., who used the same PMMA/MMA system and bioactive filler silanized with γ-methacryloxypropyltrimethoxysilane [19]. They developed PMMA-based composite cement containing glass beads, AW–glass ceramic or hydroxyapatite fillers at 70 wt%, and reported their affinity indices and mechanical properties. ST60c has a comparable affinity index to cement containing AW–glass ceramic or hydroxyapatite fillers at 70 wt%, though ST60c was inferior to cement containing glass bead at 60–70 wt%. Shinzato et al. also investigated the effect of glass bead filler size on the mechanical properties and osteoconductivity of cements containing glass beads (GBC), and reported that there was a trend for the osteoconductivity of GBC to increase as the mean glass bead size decreased [20]. We hypothesize that this trend could favorably affect the osteoconductivity of cements containing nano-sized titania. From the present study, it was not possible to predict whether the nano-size of titania particles positively affect osteoconductivity of cement. Clearly, to determine this it is necessary to compare osteoconductivity among cements containing different sized titania particles. PMMA-based composite cement containing titania particles has inferior

bending strength compared to that of cement containing glass bead filler, although our data could not be directly compared with others', as the method of measurement reported by Shinzato et al. was different [19,20,23]. It was reported that an increasing trend in bending strength was observed as the filler size in PMMA-based composite cement decreased [20]. However, our data suggested that the nano-size of titania particles did not favorably affect the mechanical properties of the cement, and this was perhaps because titania particles formed aggregates in the cement.

Indeed, the cements containing titania particles did not reach the minimum bending strength required in the ISO 5833 standard (50 MPa). However, the standard is applied to acrylic resin cements used for prosthesis fixation, and not for cements used for vertebroplasty. The optimal mechanical properties of bone cements for vertebroplasty have not yet been fully defined [33], and recently favorable vertebroplasty results using calcium phosphate cements have been reported [34]. The compressive and bending strengths of ST50c or ST60c were acceptable when compared with those of calcium phosphate-based cements used for vertebroplasty. For older patients undergoing surgery, early weight bearing on the operated site is of great significance because long-term bed rest is likely to reduce muscle strength and cause systemic side effects such as dementia, decubitus, and cardiopulmonary dysfunction. Therefore, it seems reasonable that PMMA, which sets and gains maximum strength quickly, has been used for vertebroplasty.

Titania-containing cements have been developed to replicate the beneficial characteristics of PMMA and overcome the disadvantages of PMMA [3,9–11]. PMMA bone cement was not primarily introduced as a bone substitute for vertebroplasty, but for implant fixation in orthopedic applications. Therefore, to use commercial PMMA bone cements as a bone substitute for vertebroplasty, it is

necessary to add contrast materials to improve radiopacity and visual control [35]. On the other hand, titania is an oxidized metal and a highly radiopaque material. Therefore cements containing more than 50 wt% titania are expected to be highly radiopaque. Moreover, for TiO_2-containing cements, toxicity to living tissue is diminished through the decrease in MMA monomer content, decreasing the temperature of the exothermic polymerization reaction, and the lack of leached ions. The maximum surface temperatures during polymerization, measured using an infrared thermometer in the conditions of 36.5 °C and 100% relative humidity, were 89 °C for ST60 and 126 °C for PMMAc.

5. Conclusion

We compared three types of titania-containing cement with PMMA bone cement, and found that osteoconductivity and compressive strengths of the cements containing silanized titania powder were superior to the cement containing nonsilanized titania. Moreover, the cement containing 60 wt% silanized TiO_2 showed better osteoconductivity than PMMA alone and cement containing 50 wt% silanized TiO_2.

To date, ST60c is the most acceptable TiO_2-containing cement, with good prospects for clinical use due to its good osteoconductivity and handling properties, and acceptable mechanical strength.

References

[1] Charnley J. Anchorage of the femoral head prosthesis to the shaft of the femur. J Bone Joint Surg Br 1960;42-B:28–30.

[2] Goldring SR, Schiller AL, Roelke M, Rourke CM, O'Neil DA, Harris WH. The synovial-like membrane at the bone-cement interface in loose total hip replacements and its proposed role in bone lysis. J Bone Joint Surg Am 1983;65:575–584.

[3] Freeman MA, Bradley GW, Revell PA. Observations upon the interface between bone and polymethylmethacrylate cement. J Bone Joint Surg Br 1982;64:489–493.

[4] Hernigou P, Ma W. Open wedge tibial osteotomy with acrylic bone cement as bone substitute. Knee 2001;8:103–110.

[5] Harrington KD. The use of methylmethacrylate for vertebral-body replacement and anterior stabilization of pathological fracture-dislocations of the spine due to metastatic malignant disease. J Bone Joint Surg Am 1981;63:36–46.

[6] Alvarez L, Perez-Higueras A, Quinones D, Calvo E, Rossi RE. Vertebroplasty in the treatment of vertebral tumors: postprocedural outcome and quality of life. Eur Spine J 2003;12:356–360.

[7] Cohen JE, Lylyk P, Ceratto R, Kaplan L, Umanskyt F, Gomori JM. Percutaneous vertebroplasty: technique and results in 192 procedures. Neurol Res 2004;26:41–49.

[8] Heini PF, Walchli B, Berlemann U. Percutaneous transpedicular vertebroplasty with PMMA: operative technique and early results. A prospective study for the treatment of osteoporotic compression fractures. Eur Spine J 2000;9:445–450.

[9] Dahl OE, Garvik LJ, Lyberg T. Toxic effects of methylmethacrylate monomer on leukocytes and endothelial cells in vitro. Acta Orthop Scand 1994;65:147–153.

[10] Deramond H, Wright NT, Belkoff SM. Temperature elevation caused by bone cement polymerization during vertebroplasty. Bone 1999;25(2 Suppl):17S–21S.

[11] Leeson MC, Lippitt SB. Thermal aspects of the use of polymethylmethacrylate in large metaphyseal defects in bone. A clinical review and laboratory study. Clin Orthop 1993:239–245.

[12] Tsai TT, Chen WJ, Lai PL, Chen LH, Niu CC, Fu TS, et al. Polymethylmethacrylate cement dislodgment following percutaneous vertebroplasty: a case report. Spine 2003;28:E457–E460.

[13] Cotten A, Dewatre F, Cortet B, Assaker R, Leblond D, Duquesnoy B, et al. Percutaneous vertebroplasty for osteolytic metastases and myeloma: effects of the percentage of lesion filling and the leakage of methyl methacrylate at clinical follow-up. Radiology 1996;200:525–530.

[14] Kenny SM, Buggy M. Bone cements and fillers: A review. J Mater Sci Mater Med 2003;14:923–938.

[15] Kawanabe K, Tamura J, Yamamuro T, Nakamura T, Kokubo T, Yoshihara S. A new bioactive bone cement consisting of BIS-GMA resin and bioactive glass powder. J Appl Biomater 1993;4:135–141.

[16] Tamura J, Kawanabe K, Yamamuro T, Nakamura T, Kokubo T, Yoshihara S, et al. Bioactive bone cement: the effect of amounts of glass powder and histologic changes with time. J Biomed Mater Res 1995;29:551–559.

[17] Kobayashi M, Shinzato S, Kawanabe K, Neo M, Matsushita M, Kokubo T, et al. Alumina powder/Bis-GMA composite: effect of filler content on mechanical properties and osteoconductivity. J Biomed Mater Res 2000;49:319–327.

[18] Mousa WF, Kobayashi M, Kitamura Y, Zeineldin IA, Nakamura T. Effect of silane treatment and different resin compositions on biological properties of bioactive bone cement containing apatite-wollastonite glass ceramic powder. J Biomed Mater Res 1999;47:336–344.

[19] Shinzato S, Kobayashi M, Mousa WF, Kamimura M, Neo M, Kitamura Y, et al. Bioactive polymethyl methacrylate-based bone cement: comparison of glass beads, apatite- and wollastonite-containing glass-ceramic, and hydroxyapatite fillers on mechanical and biological properties. J Biomed Mater Res 2000;51:258–272.

[20] Shinzato S, Nakamura T, Kokubo T, Kitamura Y. Bioactive bone cement: effect of filler size on mechanical properties and osteoconductivity. J Biomed Mater Res 2001;56:452–458.

[21] Uchida M, Kim H-M, Kokubo T, Nakamura T. Apatite-forming ability of titania gels with different structures. In: Ohgushi H, Hastings GW, Yoshikawa T, editors. Bioceramics. Singapore: World Scientific Publishing; 1999. p. 149–152.

[22] Fujibayashi S, Nakamura T, Nishiguchi S, Tamura J, Uchida M, Kim HM, et al. Bioactive titanium: effect of sodium removal on the bone-bonding ability of bioactive titanium prepared by alkali and heat treatment. J Biomed Mater Res 2001;56:562–570.

[23] Shinzato S, Nakamura T, Kokubo T, Kitamura Y. A new bioactive bone cement: effect of glass bead filler content on mechanical and biological properties. J Biomed Mater Res 2001;54:491–500.

[24] Nakamura T, Kato H, Okada Y, Shinzato S, Kawanabe K, Tamura J, et al. Bone cement made of high molecular weight PMMA resin with bioactive filler showed higher bone-bonding strength than that of Bis-GMA resin and bioactive ceramic fillers. In: Giannini S, Moroni A, editors. Bioceramics. Zurich, Switzerland: Trans Tech Publications; 2001. p. 661–664.

[25] Hayashi K, Uenoyama K, Matsuguchi N, Nakagawa S, Sugioka Y. The affinity of bone to hydroxyapatite and alumina in experimentally induced osteoporosis. J Arthroplasty 1989;4:257–262.

[26] Yoshikawa M, Terada Y, Toda T. Setting time and sealing ability of alpha-tricalcium phosphate cement containing titanic oxide. J Osaka Dent Univ 1998;32:67–70.

[27] Mohsen NM, Craig RG. Effect of silanation of fillers on their dispersability by monomer systems. J Oral Rehabil 1995;22:183–189.

[28] Kokubo T, Ito S, Huang ZT, Hayashi T, Sakka S, Kitsugi T, et al. Ca,P-rich layer formed on high-strength bioactive glass-ceramic A-W. J Biomed Mater Res 1990;24:331–343.

[29] Sun JS, Liu HC, Chang WH, Li J, Lin FH, Tai HC. Influence of hydroxyapatite particle size on bone cell activities: an in vitro study. J Biomed Mater Res 1998;39:390–397.

[30] Kokubo T, Kim H-M, Kawashita M, Nakamura T. Bioactive metals: preparation and properties. J Mater Sci Mater Med 2004;15:99–107.

[31] Beatty MW, Swartz ML, Moore BK, Phillips RW, Roberts TA. Effect of microfiller fraction and silane treatment on resin composite properties. J Biomed Mater Res 1998;40:12–23.

[32] Wang M, Bonfield W. Chemically coupled hydroxyapatite-polyethylene composites: structure and properties. Biomaterials 2001;22:1311–1320.

[33] Heini PF, Berlemann U. Bone substitutes in vertebroplasty. Eur Spine J 2001;10 Suppl 2:S205–S213.

[34] Nakano M, Hirano N, Matsuura K, Watanabe H, Kitagawa H, Ishihara H, et al. Percutaneous transpedicular vertebroplasty with calcium phosphate cement in the treatment of osteoporotic vertebral compression and burst fractures. J Neurosurg Spine 2002;97:287–293.

[35] Erbe EM, Clineff TD, Gualtieri G. Comparison of a new bisphenol-a-glycidyl dimethacrylate-based cortical bone void filler with polymethyl methacrylate. Eur Spine J 2001;10 Suppl 2:S147–S152.

Figure Captions

Fig. 1. Powder X-ray diffraction of titanium dioxide powder, which contained anatase crystal as its main phase.

Fig. 2. (a) Mechanical properties of T50c, ST50c, ST60c, and PMMAc (means ± SD, $n = 5$). *Significant compared with ST50c, ST60c, and PMMAc ($p < 0.01$). **Significant compared with T50c, ST50c, and ST60c ($p < 0.01$). ***Significant compared with PMMAc ($p < 0.01$). (b) Typical stress–strain curves of bending strength (A) and compressive strength (B).

Fig. 3. Giemsa surface staining of (a) T50c, (b) ST50c, (c) ST60c, and (d) PMMAc in rat tibiae 6 weeks after implantation. (e) T50c, (f) ST50c, (g) ST60c, and (h) PMMAc in rat tibiae 12 weeks after implantation. Arrowhead, cement crack; C, cement; B, bone; I or between arrows, intervening soft tissue. Bar = 30 μm.

Fig. 4. CMR photographs of (a) T50c, (b) ST50c, and (c) ST60c in rat tibiae 12 weeks after implantation. The white line (between arrows) is usually seen around T50c, ST50c, and ST60c. C, cement; B, bone. Bar = 60 μm.

Fig. 5. SEM photographs of (a) T50c, (b) ST50c, (c) ST60c, and (d) PMMAc in rat tibiae 12 weeks after implantation. C, cement; B, bone; I or between arrows, intervening soft tissue. Bar = 10 μm.

Fig. 6. SEM and SEM-EDX photographs of ST60c ((b) shows the same area as (a)) in rat tibiae 12 weeks after implantation. SEM-EDX demonstrated the bone apposition of ST60c. Bar = 10 μm

Fig. 7. SEM-EDX analysis shows a white Ti rich layer at the rim of ST60c. Between arrows = white line.

Fig. 8. Affinity indices for all the tested cements at 6 and 12 weeks ($n = 12$).

*Significant at 6 and 12 weeks ($p < 0.01$).

**Significant at 12 weeks ($p < 0.01$).

Table 1. Composition of PMMA-based bioactive cements

Cement	Powders[a]		Liquid[b]
	TiO_2	PMMA	MMA
	wt% (vol%)	wt% (vol%)	wt% (vol%)
T50c	50 (21)	20 (27)	30 (52)
ST50c	50 (21)	20 (27)	30 (52)
ST60c	60 (28)	16 (25)	24 (47)

[a]BPO was added at 4 wt% of the MMA.

[b]DMPT was added at 2 wt% of the MMA.

Fig.1

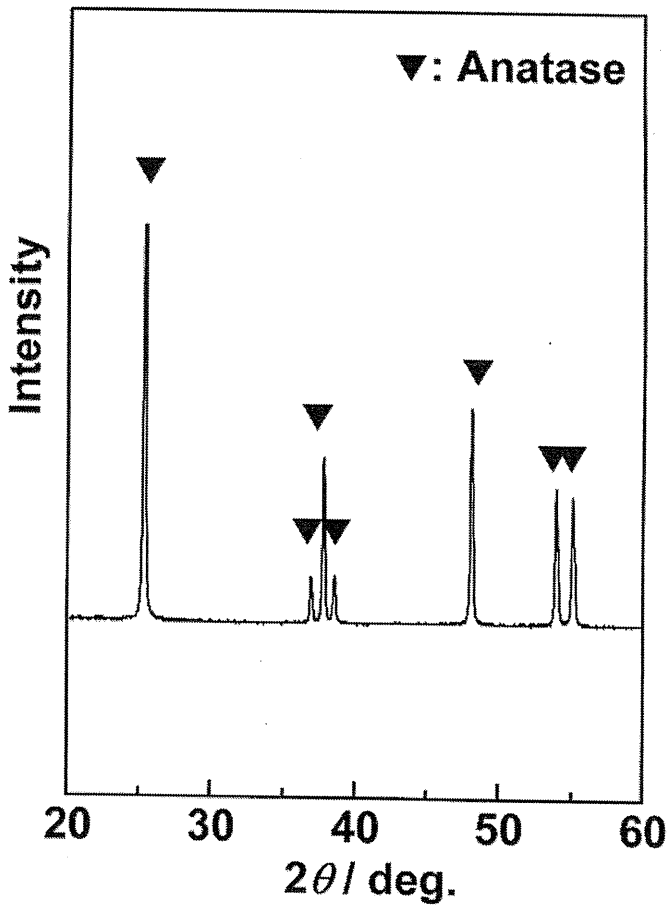


Fig.2a

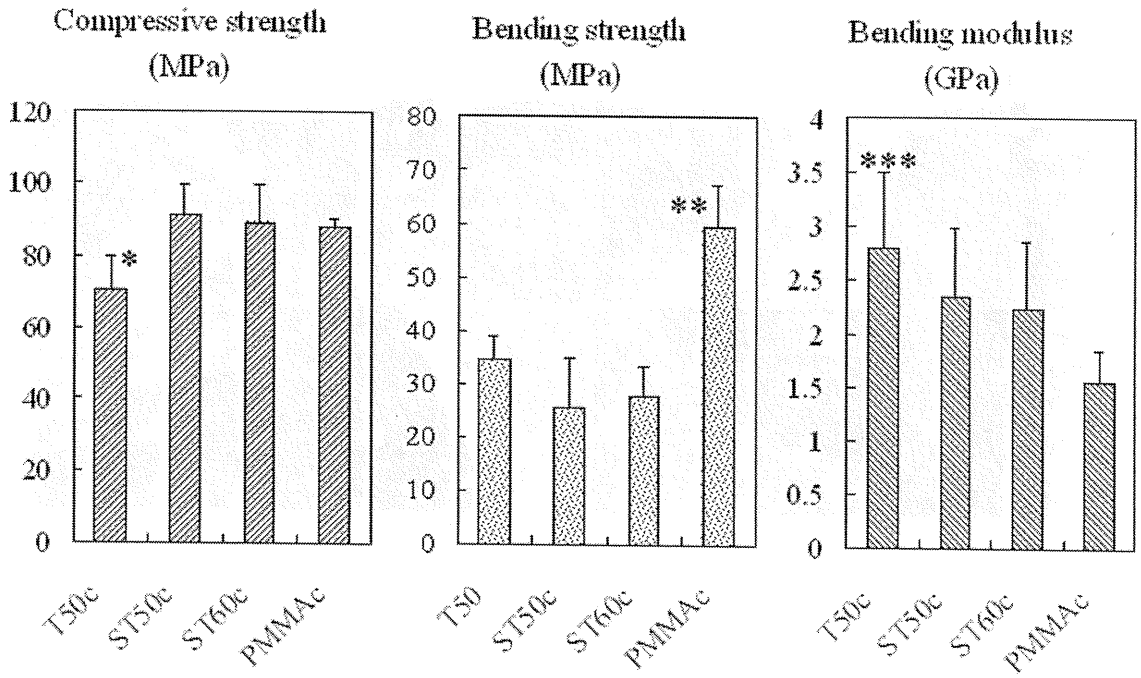

Compressive strength
(MPa)
120
100
80
60
40
20
0
*
T50c
ST50c
ST60c
PMMAc
Bending strength
(MPa)
80
70
60
50
40
30
20
10
0
**
T50
ST50c
ST60c
PMMAc
Bending modulus
(GPa)
4
3.5
3
2.5
2
1.5
1
0.5
0
***
T50c
ST50c
ST60c
PMMAc

Fig.2b

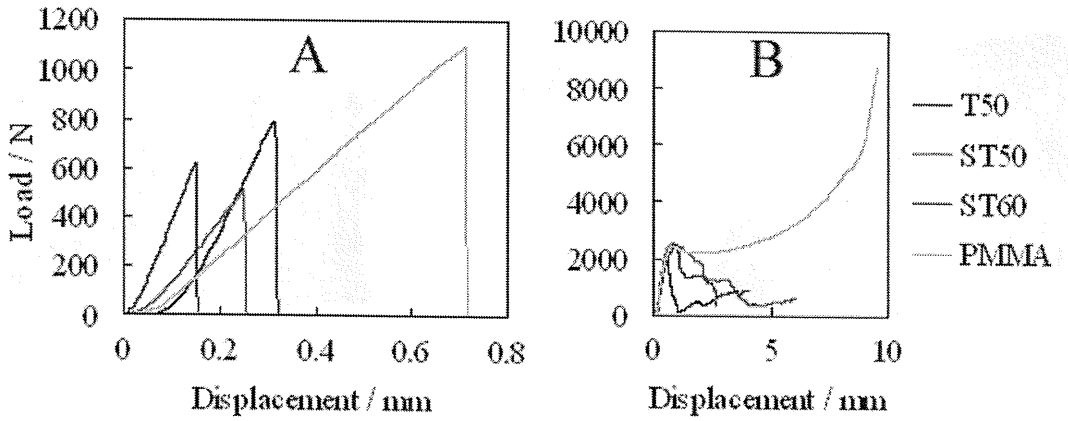

A
Load / N
1200
1000
800
600
400
200
0
0
0.2
0.4
0.6
0.8
Displacement / mm
B
10000
8000
6000
4000
2000
0
0
5
10
Displacement / mm
T50
ST50
ST60
PMMA

Fig.3

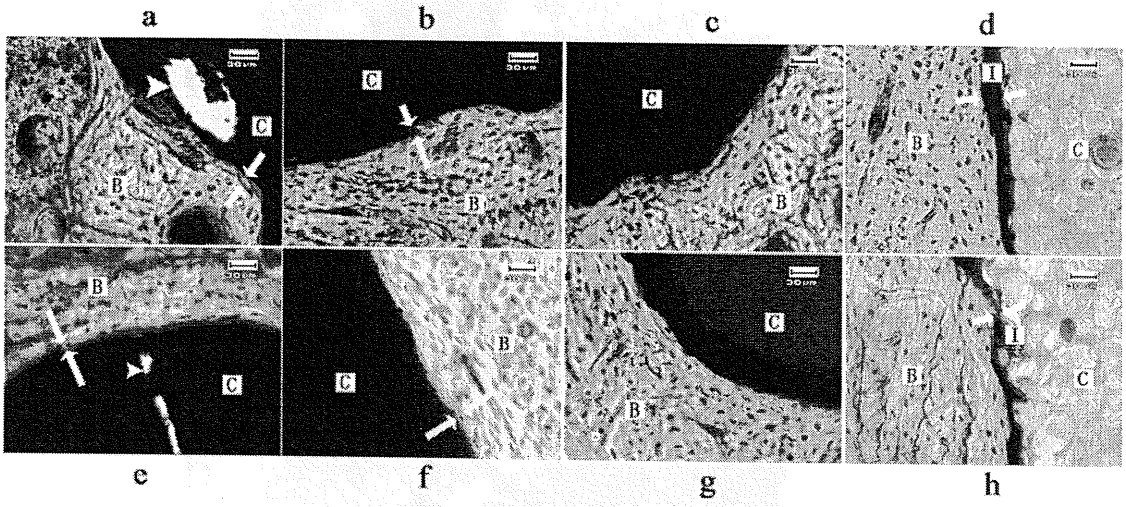


Fig.4

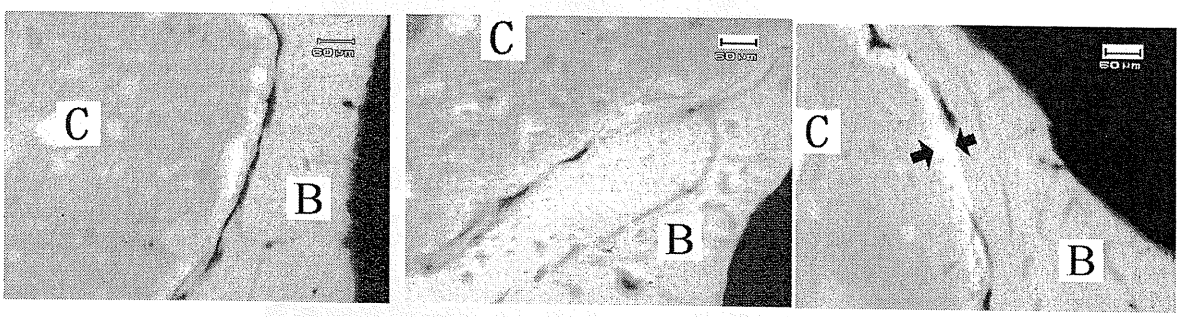
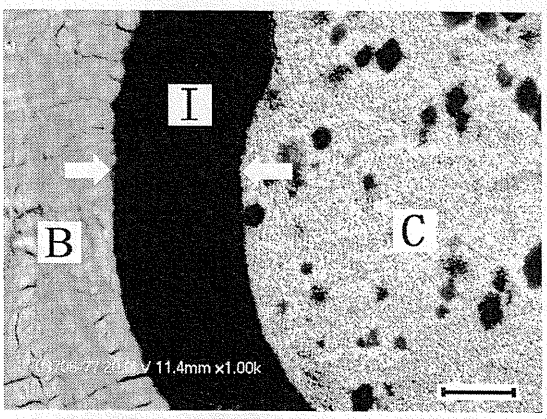
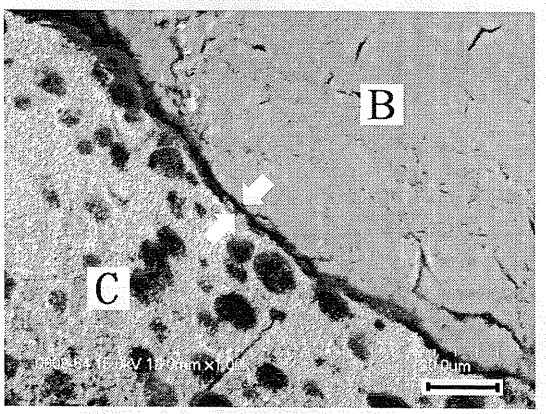


Fig.5

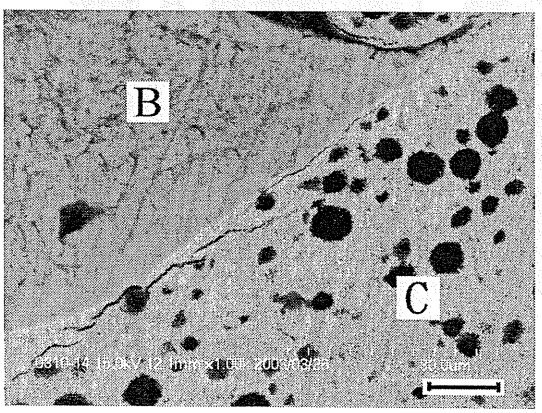
a



b



c



d

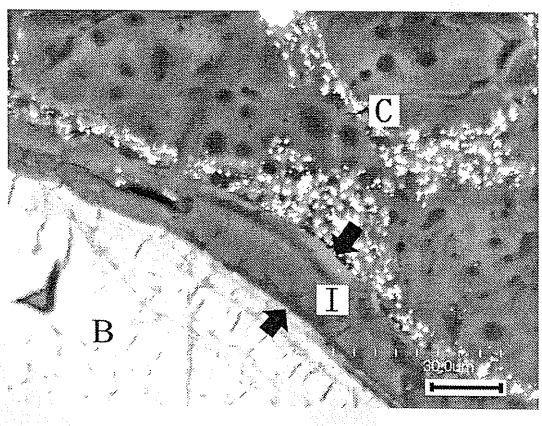


Fig.6a

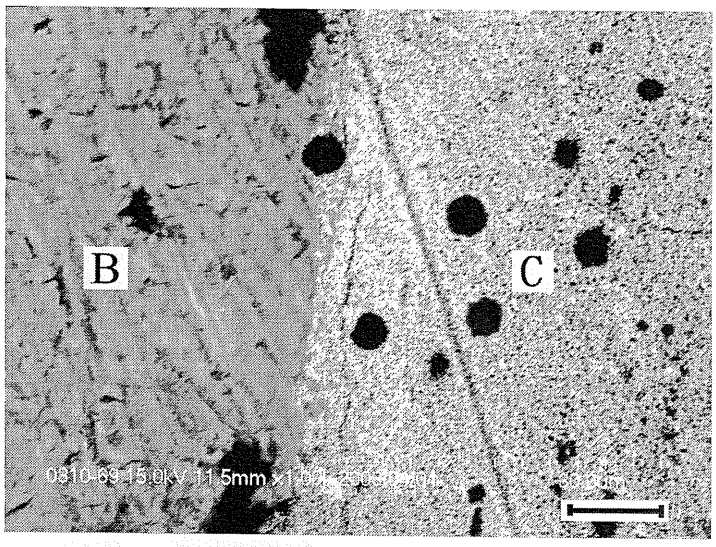


Fig.6b

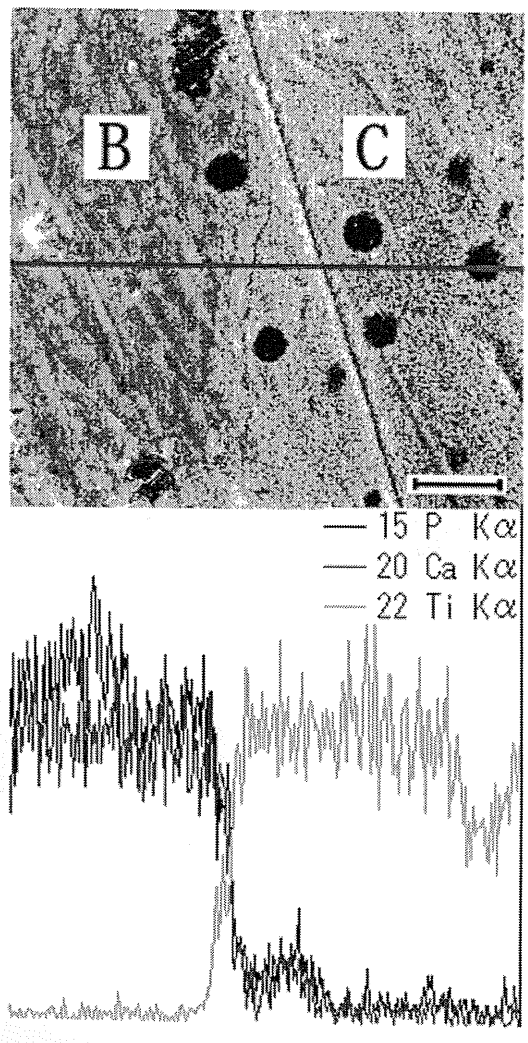


Fig.7

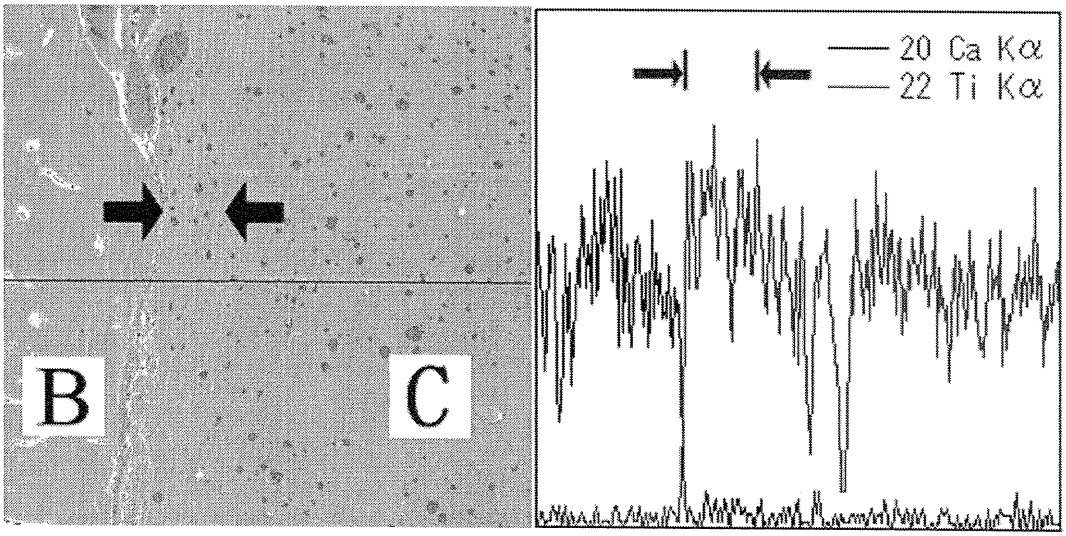
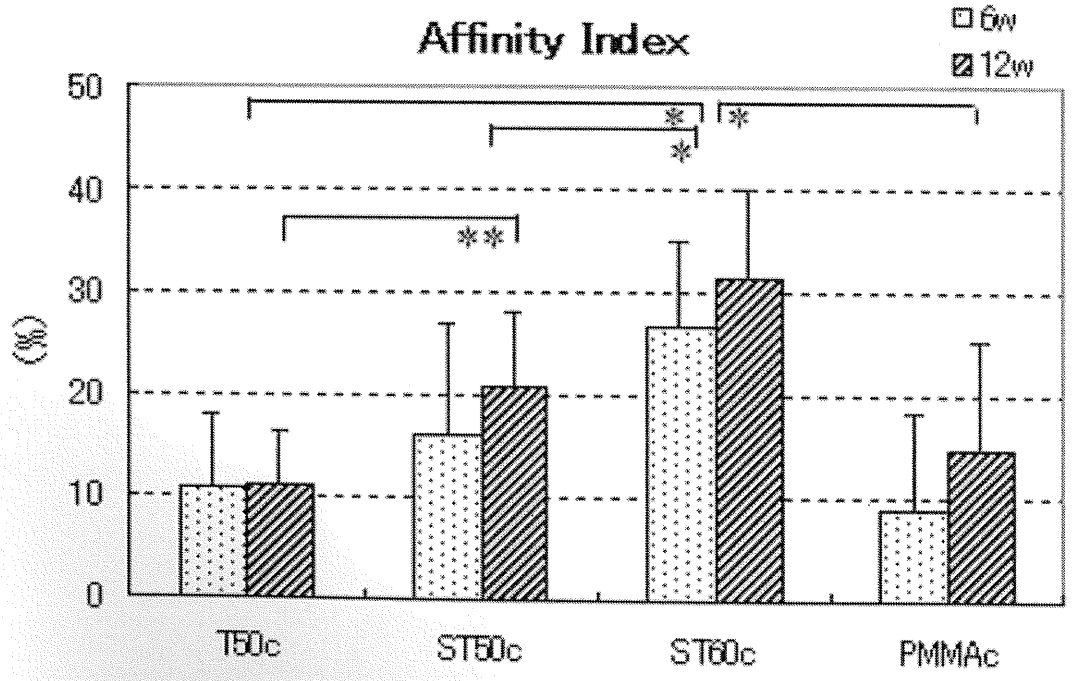

B
C
20 Ca Kα
22 Ti Kα

Fig.8


Affinity Index
6w
12w
50
40
30
20
10
0
T50c
ST50c
ST60c
PMMAc